

CLAIMS

What is claimed is:

- 1 1. A method for producing an engineered intervertebral disc tissue,
2 comprising:
3 (a) culturing intervertebral disc cells in a medium for an effective amount of
4 time to produce intervertebral disc cells surrounded by a cell-associated matrix; and
5 (b) culturing the intervertebral disc cells surrounded by the cell-associated on
6 a semipermeable membrane in the presence of one or more growth factors for a sufficient
7 amount of time to produce a coherent, engineered intervertebral disc tissue.
- 1 2. The method of claim 1 further comprising one or more of:
2 (c) isolating the intervertebral disc cells prior to (a);
3 (d) recovering the intervertebral disc cells surrounded by the cell-associated
4 matrix prior to (b);
5 (e) removing the engineered intervertebral disc tissue from the semipermeable
6 membrane; or
7 (f) implanting the engineered intervertebral disc tissue into an *in vivo*
8 intervertebral disc defect wherein the intervertebral disc tissue is implanted in the presence or
9 absence of the semipermeable membrane.
- 1 3. The method of claim 1 wherein the intervertebral disc cells are nucleus
2 pulposus or annulus fibrosus cells whereby an engineered nucleus pulposus tissue or engineered
3 annulus fibrosus tissue is produced.
- 1 4. The method of claim 1 wherein the medium of (a) is an alginate medium.

1 5. The method of claim 1 wherein the one or more growth factors is selected
2 from the group consisting of osteogenic protein-1, bone morphogenetic proteins, cartilage-
3 derived morphogenetic protein, platelet-derived growth factor, bone morphogenetic protein-2,
4 fibroblast growth factor, transforming growth factor beta, insulin-like growth factor and
5 combinations thereof.

1 6. An engineered intervertebral disc tissue produced according to the method
2 of claim 1.

1 7. The engineered intervertebral disc tissue of claim 6 wherein the tissue
2 comprises collagen, hyaluronan, proteoglycan and water.

1 8. The engineered intervertebral disc tissue of claim 7 wherein a majority of
2 the collagen comprises type I or type II.

1 9. A cohesive engineered intervertebral disc tissue comprised of greater than
2 or about 80 percent water by weight, between at or about 0.95 and 7.5 µg/mg DNA, between at
3 or about 100 and 350 µg/mg proteoglycan, and between at or about 75 and 450 µg/mg collagen,
4 wherein the DNA, proteoglycan and collagen amounts are based on the dry weight of the
5 engineered tissue.

1 10. The engineered intervertebral disc tissue of claim 9 further comprising
2 between at or about 1.5 and 3.0 µg/mg hyaluronan based on the dry weight of the engineered
3 intervertebral disc tissue.

1 11. The engineered intervertebral disc tissue of claim 9 wherein the DNA
2 content of the tissue is between at or about 3 and 4.3 µg/mg, the proteoglycan content of the
3 tissue is between at or about 100 and 200 µg/mg, the collagen content of the tissue is between at
4 or about 75 and 175 µg/mg and further wherein a majority of the collagen is type II collagen.

1 12. The engineered intervertebral disc tissue of claim 9 wherein the DNA
2 content of the tissue is between at or about 0.95 and 1.15 $\mu\text{g}/\text{mg}$, the proteoglycan content of the
3 tissue is between at or about 275 and 350 $\mu\text{g}/\text{mg}$, the collagen content of the tissue is between at
4 or about 350 and 450 $\mu\text{g}/\text{mg}$ and further wherein a majority of the collagen is type II collagen.

1 13. The engineered intervertebral disc tissue of claim 9 wherein the DNA
2 content of the tissue is between at or about 3.3 and 5.5 $\mu\text{g}/\text{mg}$, the proteoglycan content of the
3 tissue is between at or about 100 and 185 $\mu\text{g}/\text{mg}$, the collagen content of the tissue is between at
4 or about 125 and 250 $\mu\text{g}/\text{mg}$ and further wherein a majority of the collagen is type I collagen.

1 14. A method for surgically repairing intervertebral disc damage, comprising:
2 (a) producing a transplantable intervertebral disc tissue *in vitro*; and
3 (b) implanting the intervertebral disc tissue into an intervertebral disc defect.

1 15. The method of claim 14 wherein (a) comprises:
2 (i) culturing intervertebral disc cells in a medium for an effective
3 amount of time to produce intervertebral disc cells surrounded by a
4 cell-associated matrix; and
5 (ii) culturing the intervertebral disc cells surrounded by the cell-
6 associated on a semipermeable membrane in the presence of one or
7 more growth factors for a sufficient amount of time to produce a
8 coherent, engineered intervertebral disc tissue.

1 16. The method of claim 15 wherein (a) further comprises one or more of:
2 (iii) isolating the intervertebral disc cells prior to (i);
3 (iv) recovering the intervertebral disc cells surrounded by the cell-
4 associated matrix prior to (ii); and
5 (v) removing the engineered intervertebral disc tissue from the
6 semipermeable membrane.

1 17. The method of claim 15 wherein the intervertebral disc cells are annulus
2 fibrosus cells and an annulus fibrosus tissue is produced or the intervertebral disc cells are
3 nucleus pulposus tissue and a nucleus pulposus tissue is produced.

1 18. The method of claim 15 wherein the medium of (i) is an alginate medium.

1 19. The method of claim 15 wherein the one or more growth factors is
2 selected from the group consisting of osteogenic protein-1, bone morphogenetic proteins,
3 cartilage-derived morphogenetic protein, platelet-derived growth factor, bone morphogenic
4 protein-2, fibroblast growth factor, transforming growth factor beta, insulin-like growth factor
 and combinations thereof.

20. A kit for producing an intervertebral disc tissue comprising:
- (a) instructions for producing an intervertebral disc tissue; and one or more:
 - (b) growth media;
 - (c) semipermeable membranes;
 - (d) growth factors;
 - (e) one or more pieces of disposable lab equipment.